Shamblott and Gearhart Application No.: 09/767,421

Filing Da Page 2

Filing Date: January 22, 2001

AMENDMENTS TO THE CLAIMS

Please amend claims 1, 10, 15, 22, 25 and 38 as set forth below. Claims 9, 11, 12, 24, 30, 31 and 34 are canceled herein without prejudice or disclaimer.

The current listing of claims replaces all prior listings.

1. (Currently Amended) A human embryoid body derived (EBD) cell characterized by: forming disaggregated single cells upon dissociation from embryoid bodies (EB) and adhesion to defined extracellular matrix components lacking a feeder layer and lacking leukemia inhibitory factor; and having the ability to be maintained in culture on the defined extracellular matrix components in the absence of a feeder layer for at least thirty population doublings without being immortal under these conditions; and lacking detectable telomerase activity.

2-9. (Canceled)

10. (Currently Amended) The EBD cell of claim 1[[9]], wherein the EBD cells proliferate for at least sixty population doublings.

12. (Canceled)

13. (Previously presented) The EBD cell of claim 1, wherein the EBD cells are transfectable with a retrovirus or a lentivirus or both.

14. (Canceled)

15. (Currently Amended) The EBD cell of claim $\underline{1}[[9]]$, wherein the EBD cells are clonal.

16. (Previously Presented). The culture of claim 15, wherein the EBD cells are clonally derived from a single EBD cell.

Attorney Docket No. JHU1750-1

Application No.: 09/767,421

Shamblott and Gearhart

Page 3

Filing Date: January 22, 2001

17-21. (Canceled)

22. (Currently Amended) A method of obtaining a human embryoid body derived (EBD)

cell comprising:

(a) culturing primordial germ cells in a media comprising human basic fibroblast

growth factor under conditions that are suitable for formation of a solid or cystic embryoid body

having a 3-dimensional morphology;

(b) disaggregating the solid or cystic embryoid body under suitable enzymatic

conditions to provide a constituent cell or embryoid body derived (EBD) cell; and

(c) culturing the EBD cell under conditions suitable to produce a population of

proliferating EBD cells

wherein the cell is characterized as forming non-aggregated single cells upon

dissociation from embryoid bodies (EB) and adhesion to a defined substrate lacking a

feeder layer; having the ability to be maintained in culture on the defined substrate in

the absence of a feeder layer; and lacking telomerase activity and wherein the media is

selected from the group consisting of serum-free media and reduced-serum media.

23. (Previously Presented) The method of claim 22 comprising selecting a single EBD

cell from the EBD cells and culturing the single EBD cell to produce a clonal population of cells.

24. (Cancelled)

25. (Currently Amended) The method of claim [[24]] 22 comprising culturing the EBD

cell in a media selected from the group consisting of RPMI 1640 supplemented with 15% serum

[[FCS]] and media consisting essentially of hEGF, hydrocortisone, gentamicin, amphotericin-B,

fetal bovine serum, VEGF, hFGF-2, heparin, recombinant human IGF-1 and ascorbic acid.

GT\6560701.1 331323-80

Shamblott and Gearhart Application No.: 09/767,421

Filing Date: January 22, 2001

Page 4

- 26. (Previously Presented) The method of claim 25 comprising culturing the EBD cell in a media consisting essentially of hEGF, hydrocortisone, gentamicin, amphotericin-B, fetal bovine serum, VEGF, hFGF-2, heparin, recombinant human IGF-1 and ascorbic acid.
- 27. (Previously Presented) The method of claim 22 comprising culturing the EBD cell on the defined extracellular matrix components.
- 28. (Previously Presented) The method of claim 27, wherein the one or more defined extracellular matrix components are selected from the group consisting of collagen I, human extracellular matrix extract, and tissue culture-treated plastic.
- 29. (Previously Presented) The method of claim 28, wherein the one or more defined extracellular matrix components are selected from the group consisting of collagen I and human extracellular matrix.

30.-31. (Canceled)

- 32. (Previously Presented) The method of claim 22 comprising culturing the population of proliferating EBD cells for at least 30 population doublings.
 - 33.-34. (Canceled)
- 35. (Previously Presented) The method of obtaining a human EBD cell of claim 22, wherein the enzyme includes collagenase, dispase, or both.
- 36. (Previously Presented) The method of claim 22, further comprising expanding the proliferating cells on one or more defined extracellular matrix components.

In re Application of:

Shamblott and Gearhart

Application No.: 09/767,421

Filing Date: January 22, 2001

Page 5

inuary 22, 2001

37. (Previously Presented) The method of claim 36, wherein the one or more defined

Attorney Docket No. JHU1750-1

extracellular matrix components are selected from the group consisting of collagen I, human

extracellular matrix extract, and tissue culture-treated plastic.

38. (Currently Amended) A method of obtaining a human embryoid derived (EBD) cell

comprising:

(a) culturing primordial germ cells under conditions that are suitable for formation of a

solid or cystic embryoid body having a 3-dimensional morphology;

(b) disaggregating the solid or cystic embryoid body under suitable enzymatic conditions

to provide a constituent cell or embryoid derived (EBD) cell; and

(c) expanding the EBD cell under conditions suitable to produce a population of

proliferating EBD cells, wherein the EBD cells proliferate on one or more defined

extracellular matrix components, and wherein the defined extracellular matrix

components are selected from the group consisting of collagen I, human extracellular

matrix extract, and tissue culture-treated plastic,

wherein the EBD cell forms non-aggregated single cells upon dissociation from embryoid

bodies, and whereby the EBD cell will-adheres to the defined extracellular matrix

components lacking a feeder layer, and whereby the EBD cell lacks detectable

telomerase activity.

GT\6560701.1 331323-80